EFFECT OF INTRAVASCULAR COLLAGEN IN ATHEROSCLEROTIC PRIMATES.

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Aggregation of platelets at endothelial sites releases several potent vasodilator and vasoconstrictor substances. Previous studies have examined responses to these substances, but not to activation of platelets in vivo. We studied vasomotor responses of cynomolgus monkeys to activation of platelets in vivo by infusion of collagen. Purified bovine collagen (10 and 50 μg/min) was infused i.a. for 9 minutes into the perfused hind limb of normal monkeys (n=19) and monkeys fed an atherogenic diet (AS) for 19 months (n=13). Resistance of the total limb and large arteries was determined at constant flow. During the first 1-2 minutes of infusion of collagen, there was dilatation of small arteries in N and AS monkeys, probably mediated by ADP. Vasodilatation was impaired in AS monkeys ($p<0.05$ vs. N). After approximately 5 minutes of infusion of collagen, there was constriction of large arteries, which was potentiated 3-fold in AS monkeys ($p<0.05$ vs. N). Thus, when platelets aggregate in vivo, the initial response is vasodilatation, but vasoconstriction then predominates. AS impairs vasodilator responses and greatly augments vasoconstriction to platelet products, probably as a result of endothelial dysfunction. Augmented vasoconstrictor responses to aggregation of platelets in vivo may explain the propensity for vasospasm in AS, particularly when collagen is exposed at the vascular interface.

ENDOTHELIN-1 POTENTIATES CONTRACTIONS TO NOREPINEPHRINE AND SEROTONIN IN HUMAN MAMMARY AND CORONARY ARTERIES.

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Endothelin-1 is an endothelium-derived vasoconstrictor peptide. Its circulating levels are below those known to evoke direct vascular effects. To study whether low concentrations of endothelin-1 potentiate other vasoconstrictors, isolated human internal mammary artery (IMA) and left anterior decending coronary artery (LAD) were suspended in organ chambers for isometric tension recording. In IMA, the contractions to norepinephrine ($3x10^{-8}$M) were potentiated by threshold concentrations of endothelin-1 ($3x10^{-10}$M; 96±35% increase; $p<0.01$;n=6). Higher concentrations of endothelin-1 (10^{-9}M), which evoked a small contraction, further potentiated the response to norepinephrine (149±58% increase; $p<0.001$;n=6). The potentiation was prevented by the Ca^{2+} antagonist darodipine (10^{-7}M). Contractions to serotonin (5-HT; 10^{-7}M or $3x10^{-8}$M) were amplified by endothelin-1 $3x10^{-10}$M by 30±9% in IMA and 59±25% in LAD. Endothelin-1 10^{-9}M further potentiated the response (57±23% in IMA, 87±26% in LAD; $p<0.05$;n=7 and 3). The sensitivity of IMA to Ca^{2+} (10^{-7} to 10^{-3}M) was markedly enhanced by endothelin-1 ($3x10^{-10}$M; concentration shift 8-fold; $p<0.01$;n=5). In contrast to endothelin-1, 5-HT did not potentiate contractions to norepinephrine. Thus, in human arteries threshold concentrations of endothelin-1 specifically amplify contractions induced by norepinephrine and 5-HT via a calcium dependent mechanism. This may be important in vasospastic syndromes in humans.

TRANSFER OF LYSOPHOSPHATIDYLCHOLINE FROM OXIDIZED LDL TO ENDOTHELIAL CELLS IMPAIRS ENDOTHELIUM-DEPENDENT ARTERIAL RELAXATION

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Arteries from hypercholesterolemic animals and humans exhibit impaired endothelium-dependent arterial relaxation (EDR). To determine whether oxidized LDL, a lipoprotein with atherogenic properties, contributes to vasomotor dysfunction, we tested EDR of aortas isolated from normal rabbits (n=30) before and after 2 hr exposure to oxidized LDL (EC-LDL, 100 μg protein/ml) obtained by incubating native LDL with cultured endothelial cells (EC-LDL). Expressed as % of pre-incubation values, maximal EDR in response to acetylcholine, ATP, and substance P (n=6-9 each) was reduced to 7 ± 2% (SE), 39 ± 4%, and 26 ± 3%. Incubations with native LDL (up to 200 μg LDL protein/ml) had no effect on EDR. Oxidation of LDL (n=6) was accompanied by substantial hydrolysis of phosphatidylcholine to its lysoform (LPC) with a mean increase from 20 ± 5 to 590 ± 40 nmol LPC/mg LDL protein. EC-LDL (n=8) pretreated with albumin and thereby depleted of LPC (20 ± 6 nmol/mg LDL protein) became inactive like native LDL. Experiments with EC-LDL labelled with ^{14}C-LPC demonstrated wash-resistant (albumin-unextractable) transfer of LPC from EC-LDL to endothelium. Two hr incubation of arteries (n=6) with submicellar concentrations of synthetic (palmitoyl) LPC (10 μM) produced impaired EDR closely mimicking effects of EC-LDL. Thus, transferable lysophosphatidylcholine, an abundant amphiphile in atherosclerotic arteries, can account for defective endothelium-dependent relaxation induced by modified LDL.

EFFECT OF ASPIRIN ON LOCAL PROSTAGLANDIN PRODUCTION AND SEROTONIN ACCUMULATION IN A CANINE MODEL WITH CORONARY CYCLIC FLOW VARIATIONS

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We have reported that thromboxane A_2 (TxA_2) and serotonin (5-HT) are two important mediators of canine coronary cyclic flow variations (CFVs), the phenomenon caused by recurrent platelet aggregation and dislodgement on the coronary arterial wall at a site with stenosis and endothelial injury. To test the hypothesis that blocking the synthesis of TxA_2 does not affect 5-HT release, 1.1, 4.6, and 9.2 mg/kg of aspirin (ASA) were administered through the left atrium to 27 dogs. Nineteen dogs (70%) had CFVs abolished. The synthesis of TxA_2 was significantly reduced at left anterior descending coronary artery (LAD) segments with stenosis and endothelial injury. Prostacyclin production was also inhibited.

ASA Dose (mg/kg)	TxB_2 (ng/g)	6-Keto-$PGF_{1\alpha}$ (ng/g)
1.1 (n)	208 ± 36 (4)	125 ± 27 (4)
4.6 + 1.1 (n)	124 ± 31 (2)	58 ± 38 (2)
9.2 + 4.6 + 1.1 (n)	50 ± 6 (8)*	25 ± 5 (8)*

* Compared to ASA 1.1 ng/g, $p < 0.001$

However, 5-HT concentrations were not affected following all three doses of ASA, 265.7 ± 131.2 ng/g, at the LAD segment with stenosis and endothelial injury, 17.1 ± 3.7, 18.6 ± 3.7, and 19.2 ± 5.1 ng/g, at the site proximal or distal to the stenosis and normal left circumflex coronary artery, respectively. Thus, ASA eliminates CFVs in 70% of dogs and markedly diminishes TxA_2 synthesis at the site of coronary artery stenosis and endothelial injury, but does not affect 5-HT accumulation. This may help explain the additional need for a 5-HT receptor antagonist to completely eliminate CFVs in some animals receiving a TxA_2 synthase inhibitor or receptor antagonist.